

Acid Phosphatase Stimulation of the Growth of *Nocardia asteroides* and Its Possible Relationship to the Modification of Lysosomal Enzymes in Macrophages

LOVELLE BEAMAN, MARY PALIESCHESKEY, AND BLAINE L. BEAMAN*

Department of Medical Microbiology and Immunology, School of Medicine, University of California, Davis, California 95616

Received 6 August 1987/Accepted 17 February 1988

Lysosomal acid phosphatase levels are reduced in murine macrophages by virulent strains of *Nocardia asteroides*. At the same time, other lysosomal enzymes either remain unchanged or increase in activity, indicating that acid phosphatase is not lost because of degranulation or membrane leakage. This study shows that acid phosphatase was utilized as a sole carbon source by *Nocardia asteroides* and that acid phosphatase combined with glutamate as a carbon source enhanced nocardial growth. As a consequence, the inverse relationship that was observed between acid phosphatase activity and the bactericidal capacity of macrophages infected with *N. asteroides* appears to be due to the ability of *N. asteroides* to preferentially metabolize this lysosomal enzyme during growth within phagocytes.

It has been demonstrated that the level of lysosomal acid phosphatase activity in infected macrophages is inversely related to the number of intracellular cells of virulent *Nocardia asteroides* (6-9). As a consequence, a reduction in acid phosphatase activity appears to be an effective marker of the ability of these phagocytes to kill *N. asteroides* (7). Furthermore, the loss of acid phosphatase activity is not due to either degranulation or lysosomal membrane leakage, since lysozyme levels remain constant and since neutral protease-nonspecific esterase activity increases in infected macrophages at the same time that acid phosphatase decreases (9). The present study determined the probable cause of the loss of acid phosphatase activity in macrophages containing cells of *N. asteroides* that grow within these phagocytes.

N. asteroides GUH-2 and 10905 were grown in brain heart infusion broth as described previously (3, 4, 6, 8). The ability of these cells to utilize acid phosphatase as a sole source of carbon and nitrogen was determined by the methods of Gordon and Mihm (12, 13). Mineral salts agar slants containing potassium phosphate, magnesium sulfate, calcium chloride, Noble agar, and 0.5% acid phosphatase (either type I or type II) as the sole source of carbon and nitrogen were inoculated with *N. asteroides* cells washed in sterile saline (0.9%). The tubes were incubated for 28 days at 37°C, and the presence of visible growth was determined. Both *N. asteroides* GUH-2 and 10905 grew on the surfaces of slants containing acid phosphatase type I or type II. These results indicate that *N. asteroides* utilizes acid phosphatase as a carbon and nitrogen source. The utilization of acid phosphatase as compared with the utilization of lysozyme and glutamate by *N. asteroides* GUH-2 was studied further.

The growth of *N. asteroides* GUH-2 in Stanier mineral salts medium (MSM) (15) with different sources of carbon was quantitated at 37°C with rotary agitation (150 rpm). Acid phosphatase type II (Sigma Chemical Co.) was dialyzed against two changes of phosphate-buffered saline (pH 7.2) at 4°C overnight (1 g in 10 ml of phosphate-buffered saline against 1 liter of phosphate-buffered saline). The dialyzed

enzyme was filter sterilized, the purity was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and the acid phosphatase was added to Stanier MSM to yield a final concentration of 0.25 g of enzyme per 50 ml of medium in a 250-ml Erlenmeyer flask. Other 250-ml flasks containing 50 ml of Stanier MSM were prepared to contain either 0.5% monosodium glutamate (Nutritional Biochemicals) or 0.5% lysozyme (Sigma). A single-cell suspension of *N. asteroides* GUH-2 grown in brain heart infusion broth for 24 h was prepared as described previously (4). The cells were centrifuged at $1,000 \times g$ for 10 min and washed two times in Stanier MSM, and the suspension was adjusted to an A_{580} of 0.2. This suspension (1 ml) was added in triplicate to flasks containing glutamate, acid phosphatase, lysozyme, or no carbon source. At zero time, 24 h, 48 h, 6 days, 13 days, and 26 days, growth was quantitated by three measurements: CFU, filament length of the nocardial cells, and dry weight.

During the 26-day incubation the cells in medium containing acid phosphatase or lysozyme remained as single-cell suspensions, whereas in medium containing glutamate, the cells grew as large masses of clumps. There was no detectable growth in MSM without a carbon source added. Therefore, plate count assays were useful only for medium containing acid phosphatase or lysozyme, while dry-weight determinations were necessary to quantitate growth in medium containing glutamate. The growth curves of *N. asteroides* GUH-2 utilizing either lysozyme or acid phosphatase as a carbon source are presented in Fig. 1.

Since *N. asteroides* GUH-2 utilized glutamate and acid phosphatase but not lysozyme, the ability of acid phosphatase to stimulate growth in the presence of glutamate was studied. Acid phosphatase (40 mg/liter) was added to MSM containing glutamate (0.5%), and the effect on nocardial growth was quantitated (Fig. 2 and 3). Figure 2 shows filament length, and Fig. 3 shows dry-weight determinations during growth. Both the dry weight and filament length demonstrated a significant synergistic enhancement of nocardial growth in the presence of acid phosphatase as compared with growth in minimal medium containing glutamate or acid phosphatase alone. Filament length and dry-

* Corresponding author.

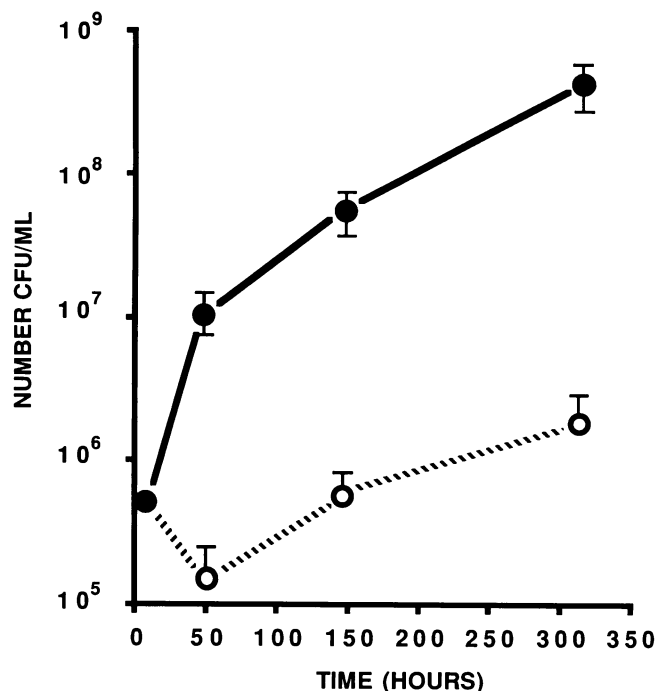


FIG. 1. Growth of *N. asteroides* GUH-2 in Stanier MSM with either acid phosphatase or lysozyme as the sole source of carbon. Symbols: ●, MSM plus 0.5 g of acid phosphatase type II per 100 ml; ○, MSM plus 0.5 g of lysozyme (egg white, grade 1; Sigma) per ml. Bars represent standard deviations.

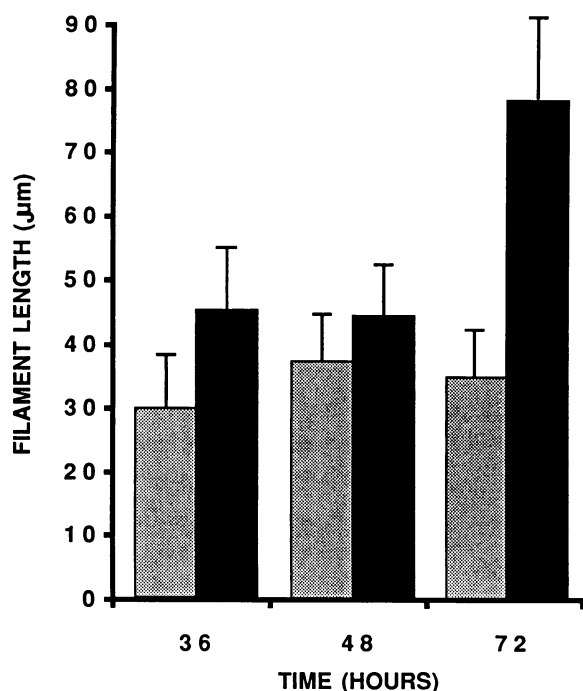


FIG. 2. Effect of acid phosphatase on the growth of *N. asteroides* GUH-2 as measured by filament length. Symbols: ▨, MSM plus glutamate; ■, MSM plus glutamate plus 40 mg of acid phosphatase type II per liter. At each time point the lengths of 10 filaments were measured and the average was calculated. Bars represent standard deviations.

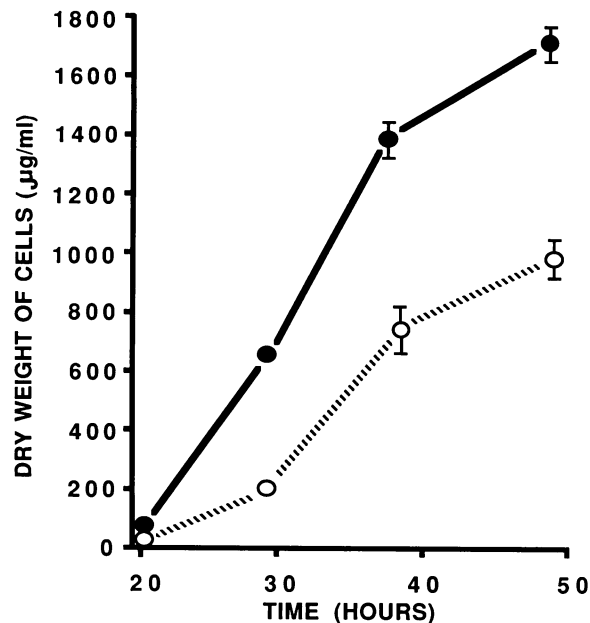


FIG. 3. Effect of acid phosphatase on the growth of *N. asteroides* GUH-2 as determined by dry weight. Symbols: ●, MSM plus glutamate plus 40 mg of acid phosphatase type II per liter; ○, MSM plus glutamate. Each point represents the average of three samples. Bars represent standard deviations.

weight analyses demonstrated that the growth rate for *N. asteroides* GUH-2 was more than doubled in the presence of 40 mg of acid phosphatase per liter (Fig. 2 and 3) as compared with growth in glutamate alone.

Mice are more susceptible to strains of *N. asteroides* that grow within macrophages than to strains that can be killed by these phagocytes (7, 11). Furthermore, virulent *Nocardia* species alter macrophage function by inhibiting phagosome-lysosome fusion (10), neutralizing acidification of the phagosomes (8), and altering lysosomal enzyme activity (6, 7, 9). The specific mechanisms involved in these modifications of phagocytic functions are not known. However, the decrease in acid phosphatase activity within macrophages is probably due to the utilization of this enzyme by *N. asteroides*. These results support the results of previous studies (4, 5) showing that acid phosphatase levels in macrophages can be used as a marker for nocardial virulence because of the degradation of this enzyme by *N. asteroides* cells that survive host bactericidal mechanisms. This decrease in acid phosphatase levels in macrophages was detectable within 3 h despite the relatively long lag phase of *N. asteroides*. This rapid effect on acid phosphatase activity may be the result of its ability to enhance nocardial growth. In addition, acid phosphatase could be used as a carbon source, and it was probably degraded in macrophages infected by virulent *N. asteroides* cells that were able to grow within these phagocytes. Therefore, another mechanism must be involved in the concurrent increase in neutral protease-nonspecific esterase activity in macrophages. Also, the rapid unimpeded growth of nocardiae in the brain (1, 2) may be related to high acid phosphatase levels in this organ (14).

This investigation was supported by Public Health Service grants 1R01-AI-20900 and AI-19579 from the National Institute of Allergy and Infectious Diseases.

We thank Sharon Anderson for typing this manuscript.

LITERATURE CITED

1. Beaman, B. L., M. E. Gershwin, A. Ahmed, S. M. Scates, and R. Deem. 1982. Response of CBA/N \times DBA/2F₁ mice to *Nocardia asteroides*. *Infect. Immun.* **35**:111-116.
2. Beaman, B. L., M. E. Gershwin, S. M. Scates, and Y. Ohsugi. 1980. Immunobiology of germfree mice infected with *Nocardia asteroides*. *Infect. Immun.* **29**:733-743.
3. Beaman, B. L., E. Goldstein, M. E. Gershwin, S. Maslan, and W. Lippert. 1978. Lung response of congenically athymic (nude), heterozygous, and Swiss Webster mice to aerogenic and intranasal infection by *Nocardia asteroides*. *Infect. Immun.* **22**:867-877.
4. Beaman, B. L., and S. Maslan. 1978. Virulence of *Nocardia asteroides* during its growth cycle. *Infect. Immun.* **20**:290-295.
5. Beaman, B. L., and M. Smathers. 1976. Interaction of *Nocardia asteroides* with cultured rabbit alveolar macrophages. *Infect. Immun.* **13**:1126-1131.
6. Black, C. M., B. L. Beaman, R. M. Donovan, and E. Goldstein. 1983. Effect of virulent and less virulent strains of *Nocardia asteroides* on acid phosphatase activity of alveolar and peritoneal macrophages maintained *in vitro*. *J. Infect. Dis.* **148**:117-124.
7. Black, C. M., B. L. Beaman, R. M. Donovan, and E. Goldstein. 1985. Intracellular acid phosphatase content and the ability of different macrophage populations to kill *Nocardia asteroides*. *Infect. Immun.* **47**:375-383.
8. Black, C. M., M. Paliescheskey, B. L. Beaman, R. M. Donovan, and E. Goldstein. 1986. Acidification of phagosomes in murine macrophages: blockage by *Nocardia asteroides*. *J. Infect. Dis.* **154**:952-958.
9. Black, C. M., M. Paliescheskey, B. L. Beaman, R. M. Donovan, and E. Goldstein. 1986. Modulation of lysosomal protease-esterase and lysozyme in Kupffer cells and peritoneal macrophages infected with *Nocardia asteroides*. *Infect. Immun.* **54**:917-919.
10. Davis-Scibienski, C., and B. L. Beaman. 1980. Interaction of *Nocardia asteroides* with rabbit alveolar macrophages: association of virulence, viability, ultrastructural damage, and phagosome-lysosome fusion. *Infect. Immun.* **28**:610-619.
11. Filice, G. A., B. L. Beaman, J. A. Krick, and J. S. Remington. 1980. Effects of human neutrophils and monocytes on *Nocardia asteroides*: failure of killing despite occurrence of oxidative metabolic burst. *J. Infect. Dis.* **142**:432-438.
12. Gordon, R. 1966. Some strains in search of a genus—*Corynebacterium*, *Mycobacterium*, *Nocardia* or what? *J. Gen. Microbiol.* **43**:329-343.
13. Gordon, R. E., and J. M. Mihm. 1957. A comparative study of some strains received as nocardiae. *J. Bacteriol.* **73**:15-27.
14. Shantha, T. R., S. L. Manocha, G. H. Bourne, and J. A. Kappers. 1969. The morphology and cytology of neurons, p. 45-62. *In* G. H. Bourne (ed.), *The structure and function of nervous tissue*, vol. II. Academic Press, Inc., New York.
15. Stanier, R., N. J. Palleroni, and M. Doudoroff. 1966. The aerobic pseudomonads: a taxonomic study. *J. Gen. Microbiol.* **43**:159-277.